

**UNITED STATES AIR FORCE
ARMSTRONG LABORATORY**

**PRO-OXIDANT BIOLOGICAL EFFECTS
OF INORGANIC COMPONENT
OF PETROLEUM: VANADIUM AND
OXIDATIVE STRESS**

Janusz Z. Byczkowski
MANTECH GEO-CENTER JOINT VENTURE
P.O. BOX 31009
DAYTON, OH 45437

Arun P. Kulkarni
TOXICOLOGY PROGRAM, COLLEGE OF PUBLIC
HEALTH, MDC-56
UNIVERSITY OF SOUTH FLORIDA
TAMPA, FL 33612

September 1996

**Occupational and Environmental Health
Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400**

Approved for public release; distribution is unlimited.

19990316 055

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Air Force Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1996-0126

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)			2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
			August 1996	Interim Report - October 1995-August 1996
4. TITLE AND SUBTITLE			5. FUNDING NUMBERS	
Pro-Oxidant Biological Effects of Inorganic Component of Petroleum: Vanadium and Oxidative Stress			Contract F41624-96-C-9010 PE 62202F PR 7757 TA 7757A2 WU 7757A203	
6. AUTHOR(S)			8. PERFORMING ORGANIZATION REPORT NUMBER	
J. Byczkowski, and A. Kulkarni				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
ManTech Geo-Centers Joint Venture P.O. Box 31009 Dayton, OH 45437-0009			AL/OE-TR-1996-0126	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION AVAILABILITY STATEMENT			12b. DISTRIBUTION CODE	
Approved for public release; distribution is unlimited.				
13. ABSTRACT (Maximum 200 words)				
<p>Crude oil contains significant amounts of inorganic compounds of vanadium. Air Force, Army, and Navy personnel are exposed to vanadium compounds in the air, on the land, and on the sea, wherever petroleum fuel is used. Inorganic residue of fly ash resulting from combustion of some fuels may contain almost exclusively vanadium oxides. Unlike organic pollutants, vanadium is not biodegradable and it may build up in certain ecosystems to the level which may be toxic to living organisms. It has been estimated that as much as 66,000 tons of vanadium is released into the atmosphere each year. Particularly dramatic effects on the environment may result from massive incidental and/or intentional spilling of vanadium-containing crude oil into relatively confined ecosystems, as well as from massive oil burning. In addition to the vanadium exposure at the work place, the general population is also exposed increasingly to this metal, mostly as a result of increased utilization of vanadium-containing petroleum fuels. Vanadium-bearing particles may persist in the lungs for many years, raising the risk of chronic health effects. This report contains a review of the extensive literature on biochemical mechanisms of action of vanadium compounds, as well as the authors' own perspective, resulting from about two decades of laboratory research. This report provides information about adverse biological effects of vanadate, vanadium pentoxide, vanadyl, and other vanadium derivatives present in petroleum or formed during the fossil fuel combustion, on oxidative stress, cellular signal transduction, subcellular organelle functions, and on a possible interaction with polycyclic aromatic hydrocarbons and chemical co-carcinogenesis.</p>				
14. SUBJECT TERMS			15. NUMBER OF PAGES	
Petroleum	Vanadium	Oxidative stress	42	
Natural oil	Vanadate	Vanadyl	16. PRICE CODE	
Free radicals	Reactive oxygen	Literature review		
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT	
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL	

PREFACE

This report contains a review of the extensive literature on biochemical mechanisms of action of vanadium compounds, as well as the authors' own perspective, resulting from about two decades of laboratory research. This is one of a series of technical reports and publications describing results of a collaborative effort conducted by ManTech Environmental Technology, Inc., Toxic Hazards Research Unit, Located at Wright-Patterson Air Force Base, and is aimed at petroleum risk assessment.

The research was conducted under Department of the Air Force Contract No. F41624-96-C-9010. Lt Col Terry A. Childress, Director of the Toxicology Division, served as Contract Technical Monitor.

TABLE OF CONTENTS

SECTION	PAGE
1 INTRODUCTION	5
<i>1.1. Vanadium as a Main Inorganic Pollutant of Petroleum.....</i>	<i>5</i>
<i>1.2. Oxidative Stress</i>	<i>6</i>
2 PRO-OXIDANT PROPERTIES OF VANADIUM.....	9
<i>2.1. Compartmentalization of $V_{(V)}$ and $V_{(IV)}$ in the Organism</i>	<i>9</i>
<i>2.2. Generation of Free Radicals.....</i>	<i>11</i>
<i>2.3. NAD(P)H Oxidation.....</i>	<i>12</i>
<i>2.4. Depletion of Cellular Antioxidants</i>	<i>13</i>
3 EFFECTS ON MITOCHONDRIA	15
<i>3.1. Inhibition of the Respiratory Chain by $V_{(V)}$.....</i>	<i>15</i>
<i>3.2. Short-circuiting of the Respiratory Chain.....</i>	<i>16</i>
4 EFFECTS ON MICROSOMES.....	18
<i>4.1. Microsomal Lipid Peroxidation.....</i>	<i>18</i>
5 CARCINOGEN CO-OXYGENATION	19
<i>5.1. Polyunsaturated Fatty Acid Peroxidation.....</i>	<i>19</i>
<i>5.2. Benzo(a)pyrene Co-oxygenation.....</i>	<i>20</i>
6 EFFECTS ON SIGNAL TRANSDUCTION	22
<i>6.1. Inhibition of Protein Tyrosine Phosphatase.....</i>	<i>22</i>
<i>6.2. Inhibition of Receptor and Phosphoprotein Dephosphorylation</i>	<i>24</i>
<i>6.3. Nonspecific Interactions</i>	<i>24</i>
7 CONCLUSIONS	25
<i>7.1. Future Research Needs</i>	<i>25</i>
8 REFERENCES.....	26

LIST OF FIGURES

FIGURE	PAGE
1 Pro-oxidant Chemicals and Free Radicals Involved in Oxidative Stress -----	6
2 Dose-dependent Effects of Pro-Oxidants on Cellular Activities-----	8
3 Oxidation of Vanadyl Depending on O ₂ Availability -----	10
4 Vanadium Redox Cycling and Lipid Peroxidation in Microsomes-----	12
5 Mode of Action of Vanadium in Mitochondria-----	15
6 Co-oxygenation of B(a)P-7,8-diol by Vanadium-----	20
7 Dose-dependent Effects of Pervanadate on PTPase Activity-----	23

ABBREVIATIONS

AP-1 - activation protein-1;
B(a)P - benzo(a)pyrene;
BBPD - biologically based pharmacodynamics;
BBPK - biologically based pharmacokinetics;
DMNQ - 2,3-dimethoxy-1,4-naphtoquinone;
FMN - flavin mononucleotide;
GSH - reduced glutathione;
NAD(P)H - reduced nicotinamide adenine dinucleotide (and its phosphate);
NF κ B - nuclear factor κ B;
NDGA - nor-dihydroguaiaretic acid;
 O_2^- - superoxide anion radical;
PBPK - physiologically based pharmacokinetics;
PTPase - phosphotyrosyl protein phosphatase;
SH2 - src homology 2 domain, a domain binding to phosphotyrosine-containing proteins;
SOD - superoxide dismutase;
TMPD - N,N,N',N'-tetramethyl-p-phenylenediamine;
 $V_{(V)}$ - pentavalent vanadium;
 $V_{(IV)}$ - tetravalent vanadium;

SECTION 1

INTRODUCTION

1.1. Vanadium as a Main Inorganic Pollutant of Petroleum

Fossil fuels, especially petroleum (crude mineral oil), contain significant amounts of vanadium compounds (Eckardt, 1971; Sokolov, 1986). Vanadium and other trace metals, unlike organic pollutants, are not biodegradable in the environment. Therefore, inorganic vanadium compounds redistributed by human activity tend to build up in the ecosystem to levels which may be toxic to living organisms. It was estimated that as much as 66,000 tons of vanadium are released and redistributed into the atmosphere each year (Nriagu and Pacyna, 1988). Devastating effects on the environment may result from massive incidental and/or intentional burning of vanadium-containing crude oil and its spilling into the sea (Sadiq and Zaidi, 1984; Kalogeropoulos et al., 1989; Vasquez et al., 1991; Madany and Raveendran, 1992; Moeller et al., 1994). Crude oil from certain locations may be especially rich in vanadium. For example, fly ash resulting from combustion of Venezuelan oil may contain up to 80% of vanadium compounds (Hudson, 1964).

In addition to fossil fuels, some ores may contain significant amounts of this metal, and thus, occupational exposure to vanadium is quite common in modern petrochemical, mining, and steel industries (Goldsmith et al., 1976; Lees, 1980; Fisher et al., 1983; Schiff and Graham, 1984; White et al., 1987; Karimov et al., 1988, 1989, 1991; Sarsebekov et al., 1994). Vanadium is often associated with uranium ore and may contribute to the increased occupational risk to those employed in uranium mines (Paschoa et al., 1987). Huge amounts of vanadium are usually deposited in the smoke-stacks and exhaust systems of engines, boilers, and generators powered by heavy fuel oils (e.g., mazut). Vanadium may be found also in diesel fuel exhaust particles (Kleinman et al., 1977; Levy et al., 1984; Rossi et al., 1986; Pistelli et al., 1991; Todaro et al., 1991; Hauser et al., 1995). Vanadium pentoxide (V_2O_5) is the most ubiquitous vanadium compound (Troppens, 1969; Nechay, 1984), although the natural oil-fired fly ash may also contain as many as a dozen other vanadium compounds (Bowden et al., 1953).

Vanadium toxicity is a true concern for industrial workers and military personnel exposed to its compounds on land and sea. In addition to vanadium exposure at the work place (Zychlinski, 1980), the general population is also increasingly exposed to this metal (Flyger et al., 1976), mostly as a result of increased utilization of vanadium-containing natural oil (Schiff and Graham, 1984). In humans, vanadium-bearing particles may persist in the lungs for many years (Paschoa et al., 1987). Thus, among the inhabitants of U.S. cities, vanadium deposits in lungs are markedly increasing with age as a result of its accumulation (Tipton and Shafer, 1964).

Acute inhalation of dust containing high concentrations of vanadium can cause harmful health effects in humans, mostly in the respiratory tract, including lung irritation, coughing, wheezing, chest pain, runny nose, and a sore throat. No comprehensive human studies are available on the health effects of chronic exposure or the carcinogenicity of vanadium; thus, the U.S. Department of Health and Human Services and the U.S. Environmental Protection Agency have not classified vanadium as to its human carcinogenicity (Agency for Toxic Substances and Disease Registry, 1992). A focussed issue, containing invited reviews and original research papers on mechanisms and biochemical effects of vanadium, edited by Srivastava and Chiasson (1995), was published in *Molecular and Cellular Biochemistry*.

1.2. Oxidative Stress

Animal studies have shown that vanadium compounds induce oxidative stress and lipid peroxidation *in vivo* (Stohs and Bagchi, 1995). An exposure of murine C3H/10T1/2 cells in culture to as low as 5 - 20 μM $\text{V}_{(\text{V})}$ was found to induce up to 100-fold expression of the proliferal gene family, indicative of a cellular state of oxidative stress (Parfett and Pilon, 1995). Even concentrations ten times lower (0.5 - 2 μM $\text{V}_{(\text{V})}$) stimulated growth of MCF-7 cells *in vitro*. The proliferative effect of $\text{V}_{(\text{V})}$ on these cells reached a plateau at 1 μM , declined at 3 μM , and disappeared at 5 μM (Auricchio et al., 1996).

The significance and biological implications of chemically induced oxidative stress have been reviewed extensively by Byczkowski and Channel (1996). In summary, oxidative stress is a pathophysiological process in which intracellular balance between endogenous as well as exogenous pro-oxidants and antioxidants is shifted towards pro-oxidants, leaving cells unprotected from free radical attack. Ultimate cellular pro-oxidants are mostly free radicals (Fig. 1), defined as molecules or groups of atoms with one or more unpaired electrons and are capable of independent existence.

Pro-Oxidant Chemicals and Free Radicals Involved in Oxidative Stress

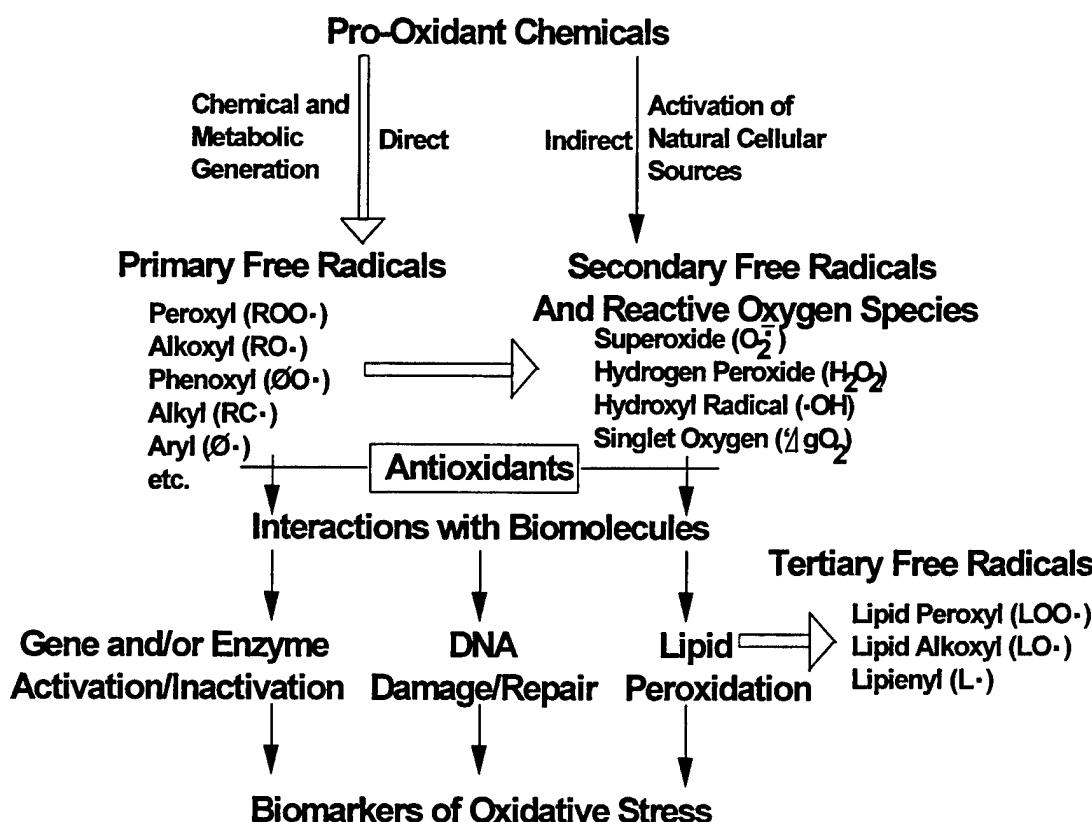


Figure 1. Generation of primary, secondary, and tertiary free radicals and reactive oxygen species involved in the oxidative stress induced by pro-oxidant chemicals (modified from Byczkowski and Channel, 1996).

These free radicals derive directly from pro-oxidant chemicals (Kehrer, 1993) or may arise indirectly from stimulated natural functions in aerobic cells (Byczkowski and Gessner, 1988); for instance, free

radicals may be produced by stimulated lipid peroxidation (Gower, 1988; Finley and Otterburn, 1993; Haegele et al., 1994). Thus, from the pro-oxidant xenobiotics, further chemical and/or metabolic reactions may generate primary free radicals (Fig. 1). Then, in an avalanche-type process, secondary free radicals and reactive oxygen species may be released (for review see Roberfroid and Calderon, 1994). Both primary and secondary free radicals may initiate lipid peroxidation, leading to the generation of tertiary free radicals (Fig. 1). Additional factors, such as aging (Stadtman et al., 1993), dietary deficiencies, or the presence of transition metals (Kulkarni and Byczkowski, 1994a, b), may augment the oxidative stress status. A depletion of cellular antioxidants such as vitamin E, C, or reduced glutathione (GSH), as well as a defective enzymatic scavenging by catalase, selenium containing GSH peroxidase, and superoxide dismutase further increase oxidative stress and may enhance damage to cellular components. Oxidative stress can be reversed by natural and synthetic antioxidants (Williams, 1993; Papas, 1993; Pratt, 1993). Oxidative stress triggers biological reactions counteracting cellular damage. It may induce enzymes with free radical scavenging and repair activities and/or activate an oxidative-stress responsive nuclear transcription factor κ B (NF κ B; Meyer et al., 1993). The importance of oxidative stress and the published literature detailing methodology employed for its measurement have been reviewed in three recent publications by Pryor (1993), Sies (1994) and Jaeschke (1995), and in a multiple author book (Spatz and Bloom, 1992).

Chemically induced oxidative stress causes derangement of antioxidant mechanisms in tissues (Videla et al., 1990), may lead to lipid peroxidation (Comporti, 1985), and may cause stimulation of cellular proliferation and/or apoptosis (Corcoran et al., 1994) that may finally result in cell injury (de Groot and Littauer, 1989). A study by Biasi et al. (1995) demonstrated that lipid peroxidation is a cause rather than an effect of necrotic tissue damage. Figure 2 summarizes dose-dependency observed in the oxidative stress induced by pro-oxidant chemicals.

Chen and Chan (1993) using 3T3-L1 cells, cultured in a serum-free medium, demonstrated that $V_{(V)}$ increases [3 H]thymidine incorporation to DNA and enhances expression of the c-fos gene in a manner analogous to a redox cycling naphthoquinone (DMNQ). The authors suggested that both pro-oxidant compounds, orthovanadate and DMNQ, increased tyrosine protein phosphorylation early in the signal transduction cascade of growth factor receptors, leading to augmentation of cell proliferation. Apparently, the common factor in the mode of action of these two completely dissimilar chemicals was an oxidative stress caused as a result of intracellular redox cycling (Chen and Chan, 1993).

Dose-Dependent Effects of Pro-Oxidants on Cellular Activities

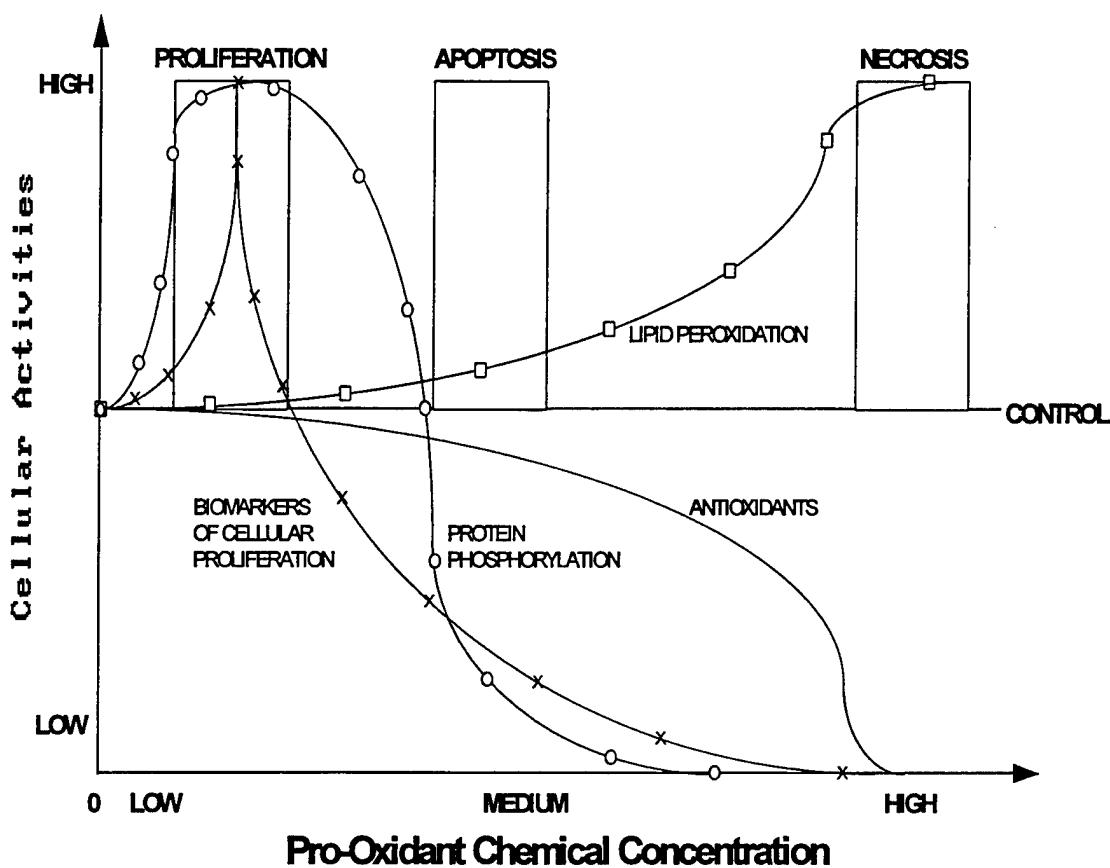


Figure 2. Effects of different concentrations of pro-oxidant chemicals on cellular function and activities:
 □—□ lipid peroxidation; —— vitamin E-type antioxidant level; ○—○ protein tyrosine phosphorylation; X—X ornithine decarboxylase and S-adenosylmethionine decarboxylase activities. The curves are computer-generated results of simulation of: lipid peroxidation and antioxidant depletion (BBPD computer program developed by Byczkowski et al., 1995, based on data by Tappel et al., 1989), protein tyrosine phosphorylation (BBPD computer program developed by Byczkowski and Flemming, 1996, based on data by Vroegop et al., 1995 and experimental results of Heffetz et al., 1990), ornithine and S-adenosylmethionine decarboxylation (BBPD computer program developed by Byczkowski and Flemming, 1996, based on Corcoran et al., 1994).

SECTION 2

PRO-OXIDANT PROPERTIES OF VANADIUM

A pro-oxidant effect of vanadium in tissue preparations was first described by Bernheim and Bernheim (1938). Since then, the biologically relevant redox properties of vanadium stirred both extensive research activity and controversy. Especially vanadium-stimulated NAD(P)H oxidation, reported for the first time by Byczkowski and Zychlinski (1978) in mitochondrial preparations, attracted much attention and became a subject of passionate discussions (Erdmann et al., 1981; Liochev and Fridovich, 1990, 1996). Because the progress in this field has been rapid, it was not possible, in the format of only one chapter, to review all of the work and cite each relevant publication. Instead, only selected experimental and review papers which we thought were key to understand and substantiate the phenomenon of oxidative stress caused by vanadium are discussed. We apologize to those whose work is not cited here.

2.1. Compartmentalization of $V_{(V)}$ and $V_{(IV)}$ in the Organism

In oxygenated blood, the absorbed vanadium circulates as poly-vanadate ($V_{(V)}$, isopolyanions containing vanadium in the +5 oxidation state). Different tissues retain vanadium mainly as vanadyl ($V_{(IV)}$, cationic form of vanadium in the +4 oxidation state) (Erdmann et al., 1984) in the presence of endogenous reducing compounds (such as glutathione-SH; Bruech et al., 1984). The highest concentration of vanadium in rats fed either $V_{(V)}$ or $V_{(IV)}$ was found in the kidneys while the liver and spleen contained about three times lower concentrations. The lowest accumulations of vanadium were found in the lung, blood plasma and blood cells. The half-life for vanadium elimination from the body was about 12 days in rats (Ramanadham et al., 1991). To date, apparently, there is no systematic pharmacokinetic study conducted on vanadium absorption, distribution, metabolism, and disposition, and no pharmacokinetic model is available describing comparative kinetics and toxicity of vanadium administered by different routes.

A successful attempt to isolate an inhibitor of (Na^+, K^+) -ATPase, inherent to the Sigma Grade commercial ATP preparations isolated from muscle, lead Cantley et al. (1977) to the discovery that sodium ortho-vanadate (Na_3VO_4 , a salt containing $V_{(V)}$) inhibits this activity at a very low concentration. In rat cardiac subcellular preparations, $V_{(V)}$ was an even more potent inhibitor of the (Na^+, K^+) -ATPase than cardiac glycoside (ouabain) which is used as a "specific inhibitor" of the sodium pump (Erdmann et al., 1984). However, a high degree of inhibition of (Na^+, K^+) -ATPase activity was observed only in the purified enzyme systems or when damaged subcellular preparations were employed. Since no evidence of inhibition was noted in intact cells or *in vivo*, it has been proposed that some kind of compartmentalization and/or "detoxification" of vanadium exists in the undamaged tissue. It was suggested that the inhibitory $V_{(V)}$ may be intracellularly reduced to vanadyl ($V_{(IV)}$) which was believed to be non-inhibitory (Erdmann et al., 1984). Inside the cell, $V_{(IV)}$ may further bind to several endogenous ligands, and be protected against reoxidation (Nechay et al., 1986). Although, as shown in the hog kidney (North and Post, 1984) and rat brain (Svoboda et al., 1984) microsomal preparations, vanadyl may also act as an inhibitor of (Na^+, K^+) -ATPase.

Using the inhibition of substrate oxidation in liver mitochondria (Byczkowski et al., 1979) as an indicator of $V_{(V)}$ accumulation, it was possible to demonstrate a significant amount of vanadate accumulatoin in the liver during experimental administration of V_2O_5 to rats (Zychlinski and Byczkowski,

1990). Although under physiological conditions, the partial oxygen pressure in liver is low (pO_2 from about 30 mm Hg in the perivenous zone to about 56 mm Hg in the periportal zone), the actual O_2 concentration in different compartments across the hepatic acinus may vary more than two-fold (Kulkarni and Byczkowski, 1994b). Due to preferential partitioning, it may be even three times higher in a lipid phase of biomembranes than in the water phase (Antunes et al., 1994). This suggests that the process of "detoxification" by reduction of $V_{(V)}$ to $V_{(IV)}$ in the rapidly perfused liver tissue may be insufficient to prevent its deleterious effects on biomembranes and bioenergetic functions, especially under conditions of chronic exposure to V_2O_5 .

These apparent discrepancies may be explained using computer simulations of vanadyl oxidation with a biologically based pharmacokinetic (BBPK) model, considering the oxygen availability (Fig. 3). The model was calibrated with the experimental data from North and Post (1984) as shown in Fig. 3C. Under conditions of chronic oxidative stress tissue reserves of GSH may be depleted. Under these circumstances, for example, 1 mM vanadyl may be completely reoxidized to $V_{(V)}$ by the available oxygen within 10 hours (Fig. 3A), provided that the tissue, stripped of antioxidants, is receiving a constant supply of oxygen (assumed to be initially no more than 124 μ M around arterial vessels in a rapidly perfused tissue).

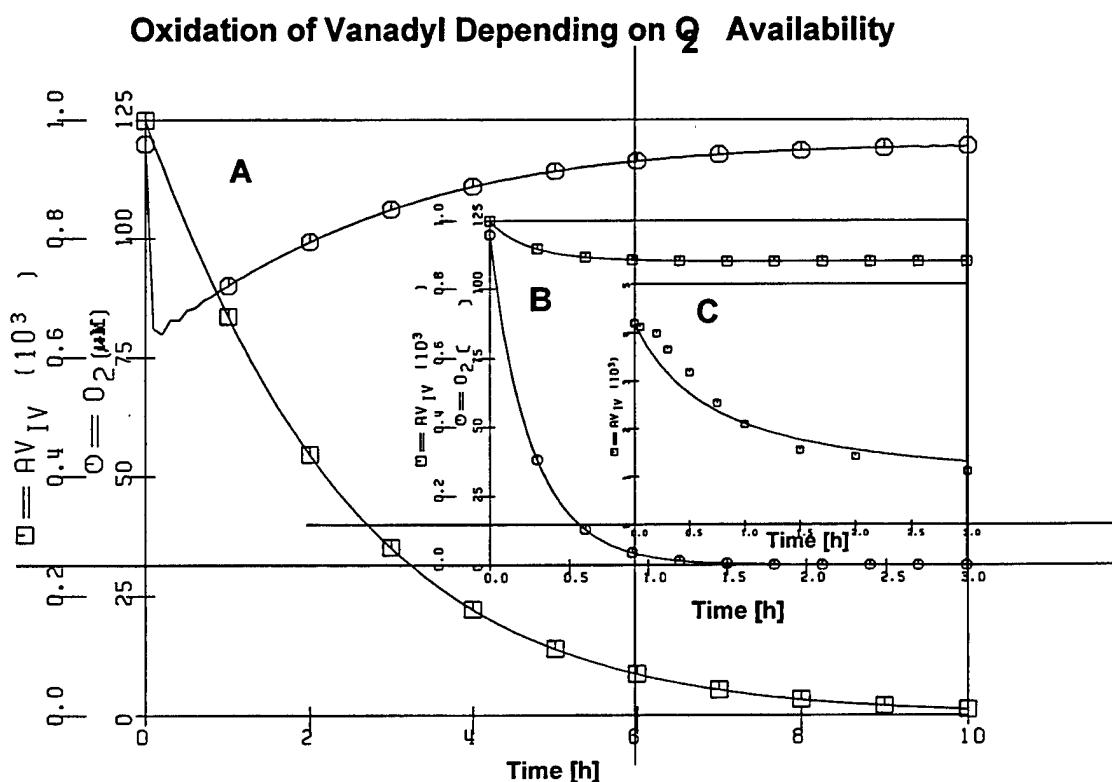


Figure 3. Oxidation of dissolved vanadyl, at near neutral pH, depending on oxygen availability. The curves are computer-generated results of simulation with biologically based pharmacokinetic model (BBPK) computer program based on North and Post, 1984; and Byczkowski et al., 1988), assuming replenishment of oxygen by diffusion from the air (C), from perfusing blood (A) or with oxygen supply turned off (B). The curves marked with small squares show vanadyl concentration (AV_{IV} [μ M]), and curves with small circles show oxygen concentration (O_2 [μ M]). The small squares in Fig. 3 C depict experimental data, from North and Post (1984), used for model calibration.

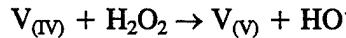
However, the same initial concentration of oxygen may be completely depleted within 1 hour in the presence of the same 1 mM vanadyl when the diffusion of fresh oxygen is restricted (Fig. 3B). This process may be drastically accelerated by the presence of traces of catalytically active transition metals, e.g., copper (Byczkowski et al., 1988). Moreover, in the perfused rat liver, $V_{(V)}$ caused significant vasoconstriction which resulted in a progressive decrease in perfusion rate (Younes and Strubelt, 1991) that was irreversible by antioxidants (Younes et al., 1991). Thus, under the conditions of reduced rate of perfusion or hypoxia, at best, up to 12% of vanadyl may be oxidized, assuming that $V_{(IV)}$ oxidation is the only process using oxygen. In reality, the tissues become anaerobic much faster (for instance, an average estimated O_2 concentration in the liver was found to be only about 35 μM ; Antunes et al., 1994), which may inhibit further reoxidation of intracellular vanadyl in poorly perfused or hypoxic tissues, even without the contribution of GSH and other cellular reductants.

2.2. Generation of Free Radicals

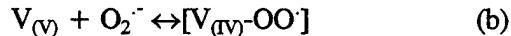
Autoxidation of $V_{(IV)}$ with O_2 from the air is a potentially deleterious, but rather a slow process, producing superoxide anion radical (O_2^-) and $V_{(V)}$:



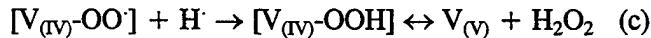
This reaction may either be drastically accelerated by the presence of catalytic amounts of transition metal cations (e.g., Cu^{2+}) or slowed down by chelators (e.g., EDTA; Byczkowski et al., 1988). Superoxide anion radicals may dismutate spontaneously or enzymatically (by superoxide dismutase, SOD), producing hydrogen peroxide (H_2O_2). It may, in turn, react with remaining $V_{(IV)}$ producing hydroxyl free radical (HO^-) in a Fenton-type reaction:



Theoretically, HO^- may react with H_2O_2 to generate O_2^- in a Haber-Weiss reaction. While the Fenton-type oxidation of $V_{(IV)}$ can easily be demonstrated under anaerobic conditions *in vitro* (Stankiewicz et al., 1991), its biological significance seems to be rather low (Byczkowski and Kulkarni, 1992b). On the other hand, in a chelating environment (Crans et al., 1989), superoxide may remain complexed within the vanadium moiety, forming a peroxy-vanadyl-type intermediate:



It seems that amino acids, peptides, and other natural ligands (analogous to Tris buffer) may stabilize the product of one-electron reduction of $V_{(V)}$ (Byczkowski and Kulkarni, 1992b). In contrast to superoxide, this hypothetical peroxy-vanadyl intermediate probably disappears very quickly under the biologically relevant conditions (Liochev and Fridovich, 1990) via hydrogen abstraction from biomolecules:



This reaction, through the pervanadate (vanadyl hydroperoxide) intermediate, may regenerate $V_{(V)}$ necessary for continuous operation of the redox cycle. The vanadium redox cycling, initiated by one-electron oxidation of vanadyl or one-electron reduction of vanadate with superoxide anion

radical, may operate as a chain reaction providing primary, secondary, and tertiary free radicals and reactive oxygen species as long as O_2^- and/or biomolecules capable of reducing $V_{(V)}$ back to $V_{(IV)}$ are available. A summary of the reactions involved in the vanadium redox cycling is shown schematically in Fig. 4.

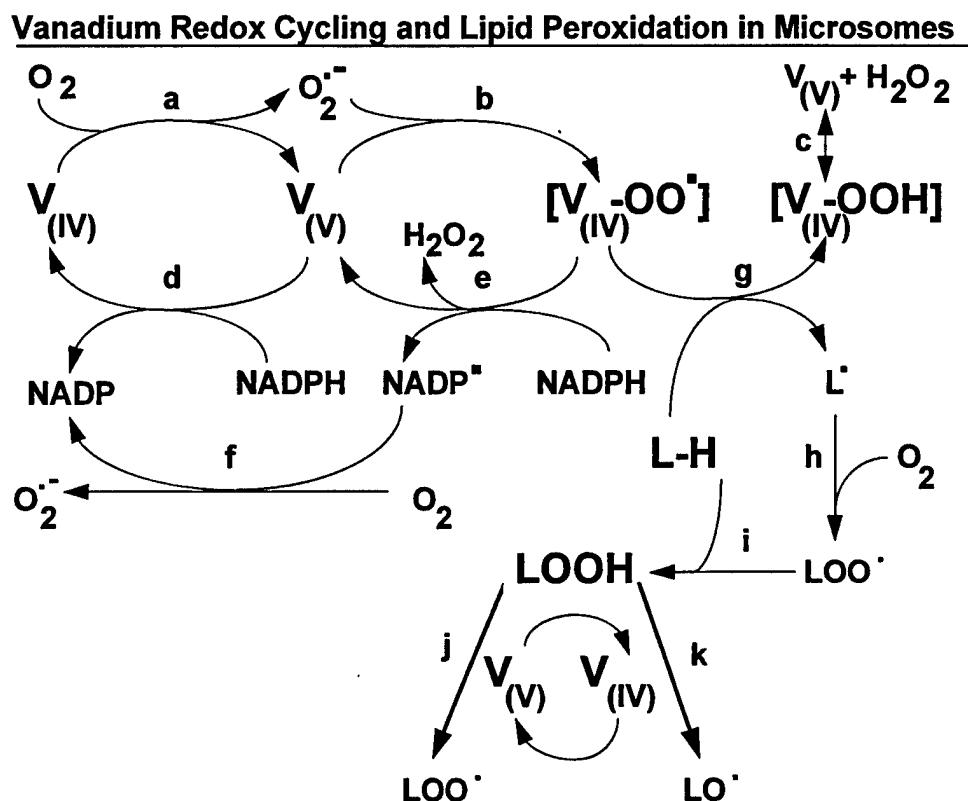
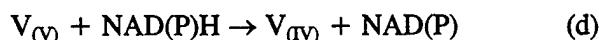


Figure 4. A summary scheme of the postulated reactions participating in vanadium redox cycling, NAD(P)H oxidation and lipid peroxidation (according to Byczkowski et al., 1988; Byczkowski and Kulkarni 1992b).

2.3. NAD(P)H Oxidation

The stimulatory effect of vanadate on oxidation of NADH was originally described by Byczkowski and Zychlinski (1978) in the sonicated rat liver mitochondrial preparations (Zychlinski and Byczkowski, 1978; Byczkowski et al., 1979). Subsequently, a similar $V_{(V)}$ stimulated NADH oxidation was reported in cardiac cell membranes (Erdmann et al., 1979) followed by many claims for NADH and NADPH oxidation in several other subcellular preparations (Ramasarma et al., 1981) and enzymatic systems (Darr and Fridovich, 1984).

Vanadate can be directly reduced by NAD(P)H at a biologically relevant pH, but this reaction is very slow (Byczkowski et al., 1979; Vyskocil et al., 1980):



In the presence of oxygen and an appropriate biological preparation, this reaction can be increased by

several hundred-fold. This reaction exhibits specific pH optima (Vyskocil et al., 1980) and is sensitive to specific inhibitors and boiling (Ramasarma et al., 1981). No wonder that many researchers thought the reaction (d) to be catalyzed by either "NAD(P)H : vanadate reductase" or "vanadium-dependent NAD(P)H oxidase". In fact, this reaction, which is insensitive to inhibition by SOD or catalase, is a "general property of endomembranes" (Ramasarma et al., 1981) containing flavin coenzymes capable of accepting electrons from NAD(P)H. Vanadate can accept electrons in a way similar to ferricyanide (Ramasarma et al., 1981). The oxidation-reduction potential for one-electron reduction of ferricyanide under standard conditions (E_0) is +0.26 V; whereas the E_0 for one-electron reduction of $V_{(V)}$ is even more positive (about +1.0 V). The biological importance of reaction (d) lies in its ability to provide reduced vanadium $V_{(IV)}$.

Under aerobic conditions, autoxidation of $V_{(IV)}$ may generate O_2^- (reaction a) which, reacting with $V_{(V)}$, may turn on a fast and destructive one-electron oxidation of NAD(P)H:



As demonstrated by Liochev and Fridovich (1988), O_2^- generated in the system containing $V_{(V)}$ and NADH initiates a free radical reaction and causes a rapid oxidation of NADH with the estimated chain length of 15 NADH oxidized per O_2^- . As expected, the overall process was inhibited by SOD (because of the reaction b), but not by catalase. Under aerobic conditions, this process may be self-sustaining, as the next step can re-generate O_2^- . Thus, in the presence of oxygen, the free radical intermediate NAD(P) $^{\cdot}$ may pass its unpaired electron on dioxygen, generating more O_2^- :



Obviously, in a well-perfused tissue, any endogenous compound (e.g., GSH) capable of reducing the initial amount of vanadate (generated endogenously or introduced) to $V_{(IV)}$ may also initiate the vanadium redox cycling and depletion of NAD(P)H. This deleterious redox cycling may be broken by anoxia or by chain-breaking antioxidants. A summary of the main reactions involved in the vanadium-mediated NAD(P)H oxidation is shown in Fig. 4.

2.4. Depletion of Cellular Antioxidants

Both water and lipid soluble antioxidants, along with enzymatic scavengers, represent the cellular defense mechanisms which allow, for instance, the liver tissue to detoxify as much as 60 - 72 μ moles O_2^- per gram liver without significant injury (Jaeschke, 1995). However, significant differences exist in the tissue levels of antioxidant protection and the ratio of water soluble vs. lipid soluble antioxidants in different species. For instance, human liver contains only 1 - 2 μ moles GSH per gram as compared to 7 - 8 μ moles GSH per gram in rat liver (Purucker and Wernze, 1990). On the other hand, physiological levels of lipid soluble antioxidants and natural free radical scavengers in a human liver are within an order of magnitude higher than in a mouse liver (Cutler, 1991; Sohal, 1993).

It was shown by Bruech et al. (1984) that exposure to $V_{(V)}$ results in the depletion of cellular thiol pool. GSH is the main intracellular thiol compound which also plays an important role as a main water-soluble cellular antioxidant. GSH is involved in the homeostasis of the intracellular redox state and is coupled to the oxidation state of cysteine residues in proteins (Ziegler, 1985). Tissue GSH was shown to participate directly in vanadate inactivation (Kretzschmar and Braunlich, 1990). As was observed *in vitro*, $V_{(V)}$ caused the oxidation of thiols, including GSH and cysteine, and the formation of thiyl radicals in

this reaction was suggested (Shi et al., 1990). Depletion of GSH not only decreases the antioxidant defense in the cytosol, but also prevents regeneration of a vital lipid-soluble antioxidant, α -tocopherol (vitamin E), increasing the vulnerability of phospholipid-rich biomembranes to oxidative stress and lipid peroxidation.

Experimental treatment of rats with V_(V) in drinking water (0.15 mg of ammonium metavanadate/mL) for 14 days resulted in a decrease in the activity of two essential "antioxidant enzymes," catalase and glutathione peroxidase, in the liver and kidneys. This decrease was linked with the increased spontaneous lipid peroxidation measured in liver and kidney homogenates. However, there was no change in the activity of cytosolic and mitochondrial superoxide dismutase (Russanov et al., 1994).

SECTION 3

EFFECTS ON MITOCHONDRIA

Experiments performed on rat liver and wheat seedling mitochondria showed that, depending on the localization of vanadate, in the intermembrane space or at the inner side of inner mitochondrial membrane, either the inhibition of the respiration with NAD-linked substrates and succinate (but not ascorbate) or short-circuiting of the respiratory chain can be observed (Fig. 5; Byczkowski et al., 1979).

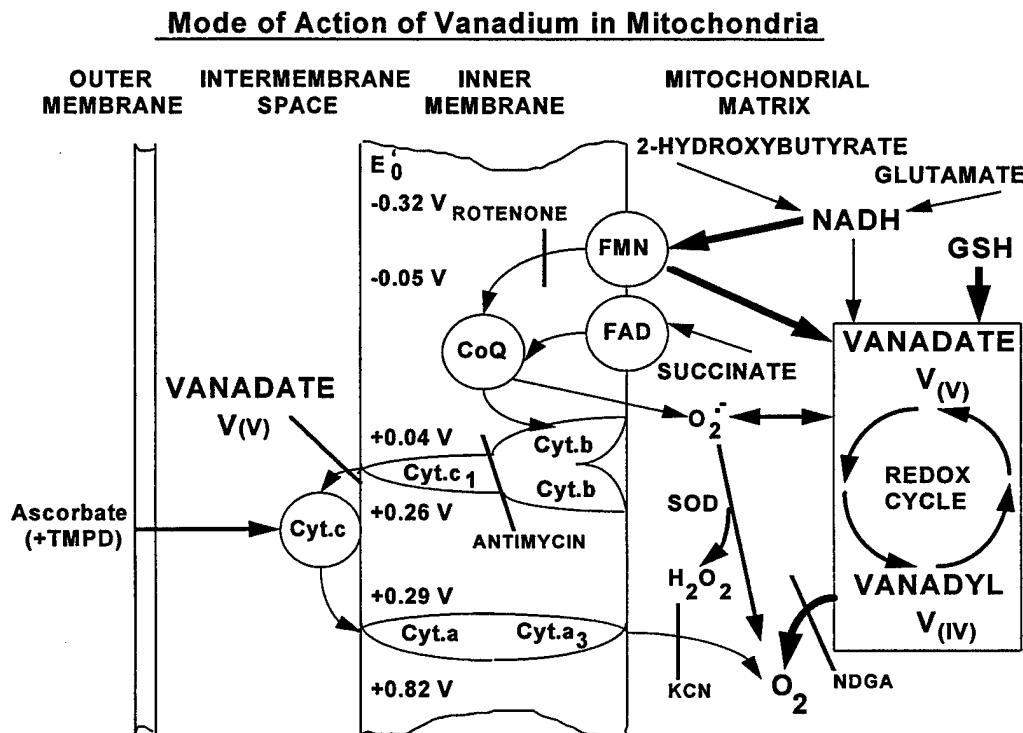


Figure 5. A summary scheme of the mode of action of vanadium in mitochondria (according to Byczkowski and Sorenson, 1984).

3.1. Inhibition of the Respiratory Chain by $V_{(V)}$

In intact mitochondria, vanadate accumulates in the intermembrane space, blocks the electron transfer through the respiratory chain between cytochrome c_1 and cytochrome c which causes inhibition of NADH-linked substrates and succinate (Byczkowski and Zychlinski, 1978; Zychlinski and Byczkowski, 1978; Byczkowski et al., 1979). In the isolated undamaged rat liver mitochondria, the respiration with glutamate and succinate at state 3 was more sensitive to inhibition by $V_{(V)}$ than at state 4, whereas the efficiency of oxidative phosphorylation (measured as ratio ADP to O) was not affected significantly up to the 0.1 mM concentration of $V_{(V)}$. Similarly, mitochondrial respiration with ascorbate (+ N,N,N',N'-tetramethyl-p-phenylenediamine, TMPD), which supplies electrons to cytochrome c , was not inhibited (Zychlinski and Byczkowski, 1990). The oxygen uptake by mitochondria isolated from the livers of rats treated intratracheally with a massive single dose of 5 mg V_2O_5 /kg was significantly inhibited for up to

48 hours with glutamate and succinate, but not with ascorbate. Even more pronounced inhibition was observed after chronic treatment of rats with 0.56 mg V₂O₅/kg monthly for 12 months (Zychlinski and Byczkowski, 1990). Other significant changes in the rats from the chronic treatment group included the decreased blood glucose and cholesterol concentrations, and increased (almost two-fold) average hydroxyproline content in the lungs (Zychlinski et al., 1991).

3.2. Short-circuiting of the Respiratory Chain

In the rat liver mitochondria damaged by sonication, V_(V) caused a dramatic stimulation of oxygen uptake with NADH-dependent substrates (Byczkowski and Zychlinski, 1978; Zychlinski and Byczkowski, 1978; Byczkowski et al., 1979). It seems that when the inner mitochondrial membrane permeability barrier to vanadate polyanions was broken, V_(V) undergoes a redox cycling. Most likely, V_(V) at the inner side of inner mitochondrial membrane is reduced by NADH at the level of flavoprotein center of the dehydrogenase (reaction "d"). Although reduction of V_(V) by NADH may proceed non-enzymatically, it is much slower than the reduction with dihydroflavin in a FMN-containing flavoprotein (Byczkowski et al., 1979). The oxidation-reduction potential of flavoprotein under standard conditions (E₀) is less negative than that of NADH (-0.05 V versus -0.32 V, respectively; Lehninger, 1965); by comparison, the E₀ for one-electron reduction of V_(V) is about +1.0 V, but may be further affected by chelation with amino acids, Tris, and other buffers.

It should be emphasized that any reaction capable of reducing V_(V) to V_(IV) (with NADH, GSH, sugars, etc.) under aerobic conditions would initiate vanadium redox cycling and eventually lead to the destructive reaction (e) and O₂[·] generation (f) (Fig. 4). Similarly, even trace amounts of O₂[·] may initiate vanadium redox cycling and produce even more O₂[·] in a chain reaction (Liochev and Fridovich, 1988). Accordingly, it was proposed that NAD(P)H oxidation in the presence of V_(V) amplifies the initial generation of O₂[·] and thus may be used as a sensitive assay method for V_(V) (Liochev and Fridovich, 1990). While the idea of O₂[·] "amplification" appears attractive, its application to the results observed with mitochondrial preparations is very difficult. First of all, mitochondria contain a highly active SOD and even in isolated submitochondrial sonic particles, washed and recentrifuged, a residual SOD activity enriched the reaction mixture with O₂ (from the dismutated O₂[·]). The additional amount of O₂ initially caused a substantial delay in the recorded oxygen uptake when compared to the NADH oxidation measured spectrophotometrically (Byczkowski, et al., 1979). Eventually, the traces of SOD became overwhelmed and, finally, destroyed by free radicals, and only then the oxygen uptake achieved its fastest rate. Moreover, when NADH oxidation was initiated by high concentrations of V_(IV), the reaction was supported by boiled mitochondria, purified cytochrome c oxidase, or even inorganic Cu²⁺ (Crane, 1975; Byczkowski and Zychlinski, 1978; Zychlinski and Byczkowski, 1978; E₀ potential for cytochrome a is +0.29 V, and for Cu²⁺ is +0.15 V).

The proposed mode of action of vanadium on mitochondria is schematically shown in Fig. 5. This scheme is based on the results of our experiments and the evidence presented by other investigators (Byczkowski and Sorenson, 1984). The overall process may be triggered either with V_(V) by one-electron reduction or with V_(IV) by one-electron oxidation. However, Liochev and Fridovich (1996) have recently proposed that the role of a biological membrane in this process is to produce O₂[·], implying that the vanadium redox cycling may be initiated only by O₂[·]. Their argument does not explain why, in intact plant mitochondria and sonicated rat liver mitochondria, the V_(V)-stimulated oxygen uptake is insensitive to rotenone with malate and/or glutamate (+ NAD). The insensitivity of this process to rotenone (which in mitochondria blocks the respiratory chain at the site before ubiquinone, and keeps flavin-dependent dehydrogenases in the reduced state but prevents the leakage of O₂[·]) rules out the involvement of O₂[·] in

the $V_{(V)}$ stimulated mitochondrial oxygen uptake at least in liver and plant mitochondria (Byczkowski et al., 1979).

SECTION 4

EFFECTS ON MICROSOMES

Analogous to the process described above for mitochondria, the addition of $V_{(V)}$ to the isolated rat hepatic or human placental microsomes, in the presence of NADPH, also evoked a significant increase in the oxygen uptake and NADPH oxidation rate (Byczkowski et al., 1988; Zychlinski et al., 1991). The experiments performed with the microsomes isolated from the human placenta showed that $V_{(V)}$ and $V_{(IV)}$ trigger lipid peroxidation due to redox cycling, increase oxygen uptake, and cause the depletion of NADPH (Byczkowski et al., 1988). Although the mechanism offered for this phenomenon was criticized by Liochev and Fridovich (1990), to date, it still remains the best possible explanation for the results obtained (Byczkowski and Kulkarni, 1992b).

4.1. Microsomal Lipid Peroxidation

In microsomes from human tissue, vanadyl and vanadate triggered lipid peroxidation due to redox cycling and formation of the reactive peroxy-vanadyl complex with superoxide (Byczkowski et al., 1988). On the basis of our experiments and the evidence presented by other workers, the following mechanism was postulated (Byczkowski et al., 1988): 1. Vanadyl_(IV) oxidizes nonenzymatically, generating $V_{(V)}$ and superoxide (O_2^-)(a); 2. $V_{(V)}$ with superoxide forms instantaneously a peroxy-vanadyl complex ($V_{(V)}-OO^-$)(b); 3. The peroxy-vanadyl complex attacks microsomal polyunsaturated lipid (L-H), abstracting hydrogen atom and initiating lipid peroxidation (g). The overall process (Fig. 4) leads to the vanadium redox cycling, consumption of O_2 , depletion of NAD(P)H, and destruction of microsomal lipids and represents a source of reactive oxygen species.

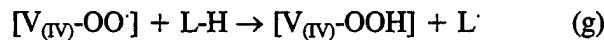
SECTION 5

CARCINOGEN CO-OXYGENATION

In several target tissues, vanadium is very likely to undergo one-electron redox cycling and initiate lipid peroxidation (Byczkowski et al., 1988). Under these prooxidant conditions, aromatic hydrocarbon co-pollutants such as benzo(a)pyrene (B(a)P), may be subjected to co-oxygenation and activation to their reactive intermediates that bind to the vital macromolecules (Dix and Marnett, 1983). This process of co-oxygenation was observed earlier by Byczkowski and Gessner (1987 a-d) during the interaction of B(a)P with asbestos and/or catalytically reactive iron in mouse liver microsomes. The results of the experiments with isolated lipoxygenase supplemented with linoleic acid suggested an involvement of lipid peroxyl and other free radical products of lipid peroxidation in activation of B(a)P-7,8-dihydrodiol to the ultimate muta- and carcinogenic epoxide (Byczkowski and Kulkarni, 1989, 1990a, 1992a). Therefore, co-occurrence of vanadium and benzo(a)pyrene as environmental pollutants raises a major concern for the possibility of synergistic interaction between them (Byczkowski and Kulkarni, 1990b).

5.1. Polyunsaturated Fatty Acid Peroxidation

In the air saturated incubation medium containing polyunsaturated fatty acid, the autoxidation of $V_{(IV)}$ initiates lipid peroxidation, analogous to that described above for microsomes (Zychlinski et al., 1991). The reaction can be partially inhibited by chain-breaking antioxidant (nor-dihydroguaiaretic acid, NDGA) and accelerated by pre-formed hydroperoxides (Byczkowski and Kulkarni, 1990b). Under identical conditions, $V_{(V)}$ was without effect. Apparently, the reactive intermediate responsible for initiation of lipid peroxidation was not a pentavalent vanadium itself, but rather its complex with O_2^- (peroxy-vanadyl):



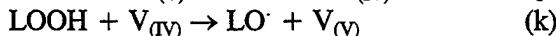
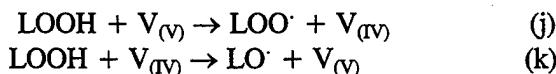
The generation of lipid-derived free radicals was sufficient to propagate lipid peroxidation in the linoleic acid system *in vitro*:



The next step in the process is expected to yield lipid hydroperoxide:



However, the endproducts of $V_{(IV)}$ -initiated peroxidation of linoleic acid did not contain linoleate hydroperoxide (Byczkowski and Kulkarni, 1992b). Therefore, it was postulated that vanadium depletes lipid hydroperoxides as shown in the following reactions:



Both reactions (j) and (k) are not dependent on superoxide ion radicals; they generate reactive lipid

alkoxyl and peroxy radicals and may further propagate lipid peroxidation (Fig. 6). It is believed that lipid-derived epoxy-peroxy radicals serve as the ultimate oxidants in the co-oxygenation of B(a)P-7,8-dihydrodiol (Hughes et al., 1989). Understanding of these reactions is crucial for explanation of the limited sensitivity of the vanadium-initiated B(a)P and its 7,8-dihydrodiol co-oxygenation to the inhibition by SOD (Byczkowski and Kulkarni, 1992b).

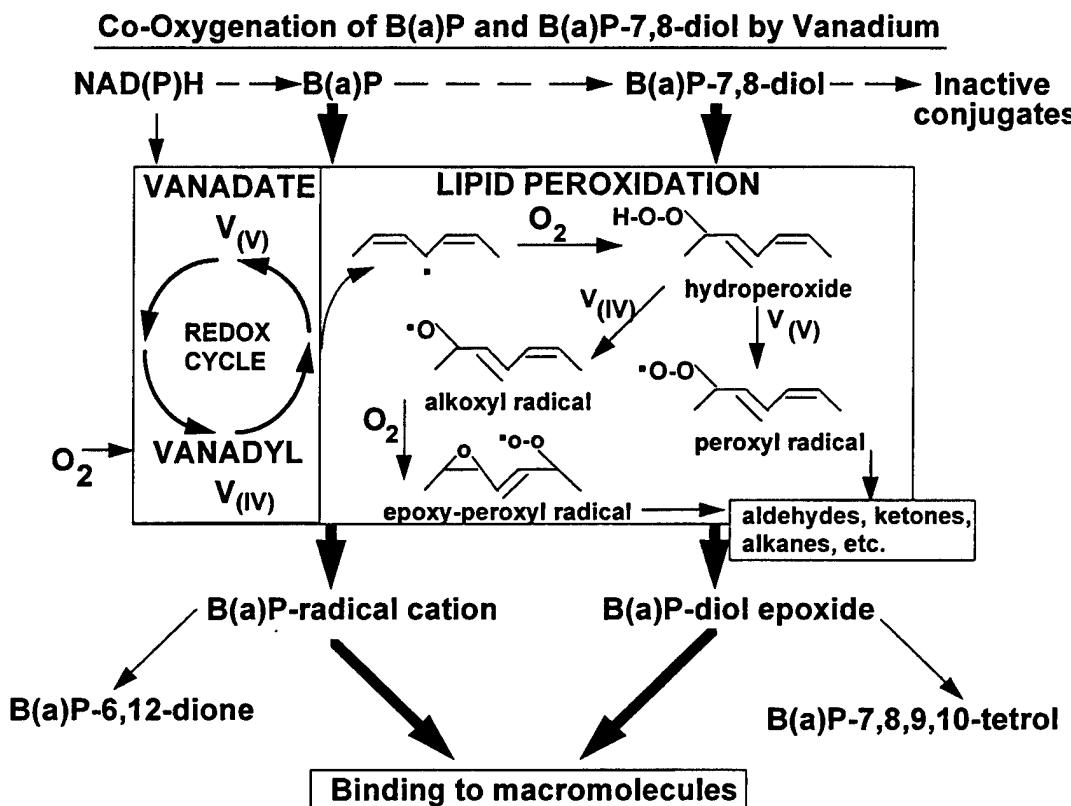


Figure 6. A summary scheme of the coupling between vanadium-initiated lipid peroxidation and benzo(a)pyrene (B(a)P) metabolism. Broken lines depict normal microsomal B(a)P metabolism, which may be inhibited by lipid peroxidation and vanadium redox cycling. Thick arrows depict activation of B(a)P and its 7,8-dihydrodiol epoxide by co-oxygenation (according to Byczkowski and Kulkarni, 1992b; 1994).

5.2. Benzo(a)pyrene Co-oxygenation

Several transition metal cations affect oxygen activation and influence the B(a)P metabolism in mammalian tissues (Byczkowski and Gessner, 1987 a,b,c,d). For instance, there is a growing body of evidence that iron-initiated lipid peroxidation may enhance the formation of the ultimate carcinogenic B(a)P metabolite (Byczkowski and Kulkarni, 1994). Byczkowski and Gessner (1987 a,c) have shown that reactive oxygen species involved in the peroxidation of hepatic microsomal lipids (reviewed by Byczkowski and Gessner, 1988) change the balance between bioactivation and conjugation of B(a)P metabolites, causing an accumulation of the B(a)P-7,8-diol epoxide and other intermediates capable of binding to macromolecules (Fig. 6). Further studies conducted on mouse liver microsomes (Byczkowski and Gessner, 1987 c,d) suggested that even the trace amount of iron present in the asbestos fibers can activate B(a)P under conditions of oxidative stress. Experimental data also showed that vanadium redox cycling effected a dose-dependent co-oxygenation of B(a)P and its 7,8-dihydrodiol thereby increasing

the generation of metabolites capable of interaction with macromolecules (Byczkowski and Kulkarni, 1992b).

SECTION 6

EFFECTS ON SIGNAL TRANSDUCTION

At the tissue level the extracellular signals conveyed by hormones, growth factors, cytokines, etc., are transmitted to the cellular nucleus by a network of protein intermediates which are sequentially phosphorylated in response to the extracellular signaling molecule. The cellular system involved in signal transduction consists of transmembrane receptors, protein kinases, phosphoprotein phosphatases, transcription factors, and the regulatory or promoter regions of genes. Several oxidants can cause deregulation of signal transduction and simultaneous activation of protein kinase C and some transcription factors (e.g., activation protein-1 or AP-1, and NF κ B; Klaassen, 1996). In this regard, it is interesting to note that the oxidative stress induced by redox cycling of naphthoquinones or orthovanadate was found to result in the activation of a membrane-associated phosphatidylinositol kinase through the tyrosine-protein phosphorylation (Chen et al., 1990).

The regulation of protein phosphorylation represents a balance between protein kinase and protein phosphatase activities (Fischer et al., 1991). The SH2 domain (src homology 2 domain) binds to phosphotyrosine residues. The SH2 domain, containing phosphotyrosyl protein phosphatases (PTPases), causes down-regulation of growth-factor receptor-initiated tyrosine phosphorylation and down-stream signal transduction pathways. The PTPases have dual specificity, hydrolyzing not only phosphotyrosyl esters, but also phosphoseryl and phosphothreonyl esters. These PTPases are involved in the regulation of cell proliferation and modulation of cell cycle (Byczkowski and Channel, 1996). Inhibition of receptor dephosphorylation and stimulation of MAP kinase activity by pro-oxidant chemicals may lead to activation of p21^{ras} and the transmission of the signal to the nucleus. This mechanism was enhanced significantly by the depletion of intracellular GSH (Lander et al., 1995).

6.1. Inhibiton of Protein Tyrosine Phosphatase

Vanadium has been shown to inhibit, *in vitro*, several enzyme systems: ATPases, adenylate kinase, phosphofructokinase, glucose-6-phosphate dehydrogenase, ribonuclease, squalene synthetase, and PTPases (see review by Nechay, 1984; and references therein). Among these, the inhibition of (Na⁺, K⁺)-ATPase by vanadate (Rifkin, 1965) has drawn the most attention. Vanadate can replace phosphate as a substrate for glyceraldehyde 3-phosphate dehydrogenase, which leads to the formation of the unstable analogue of 1,3-diphosphoglycerate (Simons, 1979). As a phosphate analog, vanadate at (sub)micromolar concentrations inhibits several other phosphatases and ATPases such as (Ca²⁺, Mg²⁺)-ATPase, (H⁺, K⁺)-ATPase, dynein ATPase, etc. (Nechay, 1984; Jandhyala and Hom, 1983; Shimizu, 1995), and thus interferes with the phosphate transfer or release reactions. On the other hand, vanadyl V_(IV) seems not to affect the (Na⁺, K⁺)-ATPase, but it inhibits alkaline phosphatase activity (Simons, 1979).

Vanadate and pervanadate (vanadyl hydroperoxide) are potent protein tyrosine phosphatase inhibitors (Fig. 7). Pervanadate may be produced, *in vitro*, in the reaction of orthovanadate with hydrogen peroxide (c) (Ianzu et al., 1990). However, in the intracellular compartments with reductive environments, it is very likely to undergo the redox cycling, yielding a family of reactive and free-radical compounds (Fig. 4). It was demonstrated that an alteration of the intracellular redox balance by decreasing the GSH levels selectively increases protein tyrosine phosphorylation, while it does not alter the serine/threonine phosphorylation (Staal et al., 1994). This effect was accounted for by selective effects on redox-sensitive

protein tyrosine phosphatase. Typically, PTPases contain nucleophilic cysteinyl residue in their catalytic center (Stone and Dixon, 1994). Their enzymatic activities are rapidly inhibited by small disulfides (Ziegler, 1985). It seems that the cysteinyl residue must be kept in the reduced -SH form, therefore, thiol-directed reagents that oxidize it cause inhibition of PTPases (Fischer et al., 1991). It is therefore possible that, at millimolar concentrations, vanadium can affect intracellular redox potential and can lead to the inhibition of PTPase activity by causing oxidation of essential cysteinyl thiols. This pro-oxidant action of $V_{(V)}$ is independent of its interference with phosphate groups and, thus, it seems that at least two independent sites of inhibition should exist within the protein tyrosine phosphatase for $V_{(V)}$ and other pro-oxidants (Hecht and Zick, 1992). Accordingly, it was suggested that $V_{(V)}$ induced the expression of two categories of genes in mouse C127 cells by two separate mechanisms (Yin et al., 1992). The stimulation of the synthesis of mRNA of c-jun was dramatically enhanced by either NADH or H_2O_2 and partly inhibited by catalase, suggesting the involvement of vanadium redox cycling, while the vanadate-stimulated synthesis of mRNA of actin and c-Ha-ras was unaffected by oxidants, reductants, or antioxidants.

Dose-Dependent Effects of Pervanadate on PTPase Activity

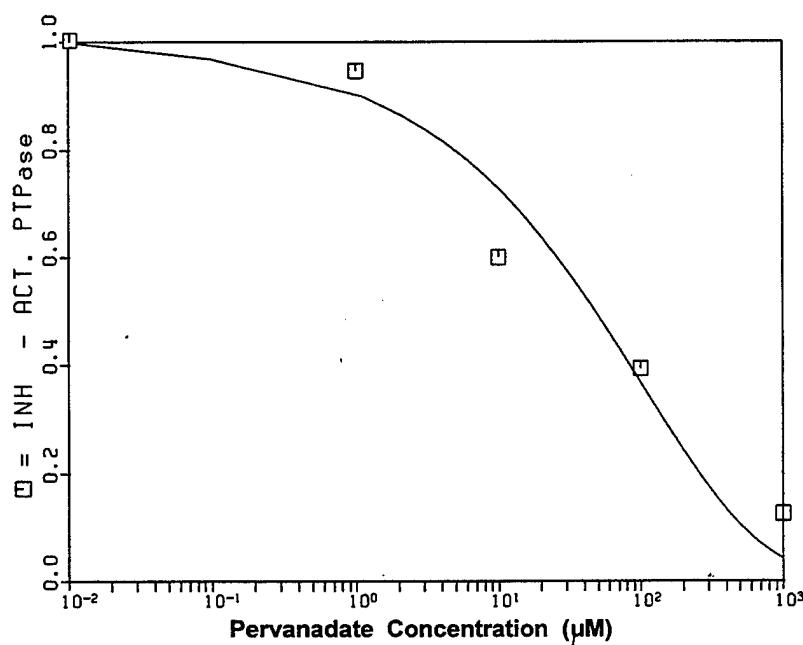


Figure 7. Dose-dependent effects of vanadate in the presence of excess H_2O_2 (pervanadate) on PTPase activity in intact rat hepatoma (Fao) cells. The curve is a computer-generated result of simulation with computer program (BBPD computer program developed by Byczkowski and Flemming, 1996, based on Heffetz et al. 1990). The small squares depict experimental data from Heffetz et al. (1990).

6.2. Inhibition of Receptor and Phosphoprotein Dephosphorylation

Increased phosphorylation of several soluble proteins and membrane receptors by vanadate and pervanadate was reported in diverse biological systems (Heffetz et al., 1990; Purushotham et al., 1995; Rokhlin and Cohen, 1995; Haque et al., 1995; Lin and Grinnell, 1995; Yamaguchi et al., 1995). Similar stimulatory effects on receptor phosphorylation status and enzymatic protein phosphorylation also were reported for other pro-oxidants (Tan et al., 1995). On the other hand, at high concentrations free radicals generated by the pro-oxidants caused nonspecific crosslinking, polymerization, and/or fragmentation of proteins, changes in membrane fluidity, and a decrease in the number of binding sites and/or binding affinity for specific ligands. In macrophages, $V_{(V)}$ reduced their ability to interact with interferon γ as well as other cytokines, while increased their basal spontaneous release of O_2^- and H_2O_2 (Cohen et al., 1996).

6.3. Nonspecific Interactions

Paradoxically, under certain conditions vanadate was shown to stimulate Ca^{2+} -ATPase from pig heart sarcoplasmic reticulum (Erdmann et al., 1984). Reportedly, toxic doses of vanadium stimulated activity of monoamine oxidase (see review by Jandhyala and Hom, 1983; and references therein) and also the secretion of pancreatic enzymes (Proffitt and Case, 1984a, b).

Vanadate, at reasonably low concentrations, enhanced adenyl cyclase activity in several cell membrane preparations, thereby increasing the levels of cyclic AMP (Erdmann et al., 1984). This mechanism seems responsible for the secretion of Cl^- , Na^+ and water in rat jejunum (Hajjar et al., 1986). However, in some isolated multicellular model systems like toad skin or bladder, vanadate inhibited effects of cyclic AMP on osmotic water flow (Nechay, 1984). Also, both vanadate and vanadyl *in vitro*, decreased cyclic AMP production in the luteinizing hormone-treated rat corpora lutea and the involvement of vanadium-stimulated tyrosine phosphorylation in this process was proposed (Lahav et al., 1986). Involvement of tyrosine kinase stimulation was also suggested in pervanadate-stimulation of respiratory burst in neutrophils (Yamaguchi et al., 1995). Therefore, it seems that stimulation of tyrosine phosphorylation of phospholipase C, activation of phospholipase C, increased production of diacyl-glycerols (DAG), and possibly, protein kinase C and/or tyrosine kinase activation (Yamaguchi et al., 1995) may be all involved in nonspecific effects of high concentrations of $V_{(V)}$ on several cellular systems, analogous to more specific action exerted, for instance, by a classical tumor promoter, phorbol acetate (Byczkowski and Channel, 1996). On the other hand, drastic oxidation of purified protein kinase C damaged both regulatory and catalytic domains (Ramasarma, 1990) which may cause inactivation of the protein kinase C-dependent signal transduction *in vivo*.

SECTION 7

CONCLUSIONS

Although in a trace amount vanadium may be essential to normal metabolic functions of the organism, excessive exposure to this element is potentially deleterious, and may even be toxic. Due to its complex chemistry and ability to undergo a free radical redox cycling, vanadium may adversely affect several biochemical functions and is capable of causing oxidative stress, co-oxygenating carcinogens, and interfering with signal transduction. The results reported in the literature are sometimes based on a treatment with a single dose or *in vitro* experiments in the presence of just one concentration of vanadium. A very few systematic dose-response studies have been conducted with vanadium to date. Clearly, more experimental data are necessary to assess fully its toxicological potential and a possible health hazard from exposure to vanadium compounds.

7.1. Future Research Needs

A systematic pharmacokinetic study should be conducted on vanadium absorption, distribution, metabolism, and disposition. The development of a pharmacokinetic/dynamic model should be very helpful to understand the kinetics and toxicity of vanadium administered by different routes (analogous to the pharmacokinetic model described for chromium by O'Flaherty, 1993, 1996). Currently, there is no information available about the microcompartmentalization and the redox state of vanadium in different subcellular fractions of the target tissues. More experimental data are necessary to understand the mode of action of vanadium on the cellular signal transduction pathway and DNA transcription as well as carcinogenicity, if any.

SECTION 8

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 1992. Toxicological Profile for Vanadium. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Antunes, F., A. Salvador, H.S. Marinho, and R.E. Pinto. 1994. A mathematical model for lipid peroxidation in inner mitochondrial membranes. *Trav. Laboratoire* 33: (suppl.T-1) 1-52.

Auricchio, F., A. Migliaccio, G. Castoria, M. Di Domenico, A. Bilancio, and A. Rotondi. 1996. Protein tyrosine phosphorylation and estradiol action. *Ann. NY Acad. Sci.* 784: 149-170.

Bernheim, F. and M.L.C. Bernheim. 1938. Action of vanadium on tissue oxidations. *Science* 88: 481-482.

Biasi, F., M. Bosco, G. Lafranco, and G. Poli. 1995. Cytolysis does not per se induce lipid peroxidation: evidence in man. *Free Radical Biol. Med.* 18: 909-912.

Bowden, A.T., P. Draper, and H. Rawling. 1953. The problem of fuel oil deposition in open-cycle gas turbines. *Proc. Inst. Mech. Eng.* 167: 291-313.

Bruech, M., M.E. Quintanilla, W. Legrum, J. Koch, K.J. Netter, and G.F. Fuhrmann. 1984. Effects of vanadate on intracellular reduction equivalents in mouse liver and the fate of vanadium in plasma, erythrocytes and liver. *Toxicology* 31: 283-295.

Byczkowski, J.Z. and S.R. Channel. 1996. Chemically induced oxidative stress and tumorigenesis: effects on signal transduction and cell proliferation. *Toxic Substance Mechan.* 15: 101-128.

Byczkowski, J.Z. and C.D. Flemming. 1996. Computer-aided dose-response characteristics of chemically initiated oxidative stress in vitro. *Toxicologist* 30: 240(1227).

Byczkowski, J.Z. and T. Gessner. 1987a. Effects of superoxide generated in vitro on glucuronidation of benzo(a)pyrene metabolites by mouse liver microsomes. *Int. J. Biochem.* 19: 531-537.

Byczkowski, J.Z. and T. Gessner. 1987b. Interaction between vitamin K3 and benzo(a)pyrene metabolism in uninduced microsomes. *Int. J. Biochem.* 19: 1173-1179.

Byczkowski, J.Z. and T. Gessner. 1987c. Action of xanthine-xanthine oxidase system on microsomal benzo(a)pyrene metabolism in vitro. *Gen. Pharmac.* 18: 385-395.

Byczkowski, J.Z. and T. Gessner. 1987d. Asbestos-catalyzed oxidation of benzo(a)pyrene by superoxide-peroxidized microsomes. *Bull. Environ. Contam. Toxicol.* 39: 312-317.

Byczkowski, J.Z., and T. Gessner. 1988. Biological role of superoxide ion-radical. *Int. J. Biochem.* 20:

569-580.

Byczkowski, J.Z. and A.P. Kulkarni. 1989. Lipoxygenase-catalyzed epoxidation of benzo(a)pyrene-7,8-dihydrodiol. *Biochem. Biophys. Res. Comm.* 159: 1190-1205.

Byczkowski, J.Z. and A.P. Kulkarni. 1990a. Activation of Benzo(a)pyrene-7,8-dihydrodiol in rat uterus: an in vitro study. *J. Biochem. Toxicol.* 5: 139-145.

Byczkowski, J.Z. and A.P. Kulkarni. 1990b. Lipid Peroxidation and Benzo(a)pyrene Derivative Co-Oxygenation by Environmental Pollutants. *Bull. Environ. Contam. Toxicol.* 45: 633-640.

Byczkowski, J.Z., and A.P. Kulkarni. 1992a. Linoleate-dependent co-oxygenation of benzo(a)pyrene and benzo(a)pyrene-7,8-dihydrodiol by rat cytosolic lipoxygenase. *Xenobiotica* 22: 609-618.

Byczkowski, J.Z. and A.P. Kulkarni. 1992b. Vanadium redox cycling, lipid peroxidation and co-oxygenation of benzo(a)pyrene-7,8-dihydrodiol. *Biochim. Biophys. Acta* 1125: 134-141.

Byczkowski, J.Z., and A.P. Kulkarni. 1994. Oxidative stress and asbestos. In J.O. Nriagu and M.S. Simmons, Eds., *Environmental Oxidants*. John Wiley and Sons, Inc. New York, pp. 459-474.

Byczkowski, J.Z., T. Pravecek, and S.R. Channel. 1995. Computer simulations of lipid peroxidation in precision cut liver slices and experimental calibration of the mathematical model. *Toxicologist* 15: 281(1504).

Byczkowski, J.Z. and J.R.J. Sorenson. 1984. Effects of metal compounds on mitochondrial function: a review. *Sci. Total. Environ.* 37: 133-162.

Byczkowski, J.Z., B. Wan, and A.P. Kulkarni. 1988. Vanadium-mediated lipid peroxidation in microsomes from human term placenta. *Bull. Environ. Contam. Toxicol.* 41: 696-703.

Byczkowski, J.Z. and L. Zychlinski. 1978. Interaction of vanadium with electron-transport chain in rat liver mitochondria in vitro. 16 Meeting of the Polish Biochem. Soc., Abstracts p. 78 (SH-4), Lodz, Poland.

Byczkowski, J.Z., L. Zychlinski, and J. Tluczkiewicz. 1979. Interaction of vanadate with respiratory chain of rat liver and wheat seedling mitochondria. *Int. J. Biochem.* 10: 1007-1011.

Cantley, L.C.Jr., L. Josephson, R. Warner, M. Yangisawa, C. Lechene, and G. Guidotti. 1977. Vanadate is a potent (Na,K)-ATPase inhibitor found in ATP derived from muscle. *J. Biol. Chem.* 252: 7421-7423.

Chen, Y. and T.M. Chan. 1993. Orthovanadate and 2,3-dimethoxy-1,4-naphtoquinone augment growth factor-induced cell proliferation and c-fos gene expression in 3T3-L1 cells. *Arch. Biochem. Biophys.* 305: 9-16.

Chen, Y.X., D.C. Yang, A.B. Brown, Y. Jeng, A. Tatoyan, and T.M. Chan. 1990. Activation of a membrane-associated phosphatidylinositol kinase through tyrosine-protein phosphorylation by

naphthoquinones and orthovanadate. *Arch. Biochem. Biophys.* 238: 184-192.

Cohen, M.D., T.P. McManus, Z. Yang, Q. Qu, R.B. Schlesinger, J.T. Zelikoff. 1996. Vanadium affects macrophage interferon- γ -binding and -inducible responses. *Toxicol. Appl. Pharmacol.* 138: 110-120.

Comporti, M. 1985. Biology of disease, lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.* 53: 599-623.

Corcoran, G.B., L. Fix, D.P. Jones, M.T. Moslen, P. Nicotera, F.Z. Oberhaimmer, and R. Buttyan. 1994. Apoptosis: molecular control point in toxicity. *Toxicol. Appl. Pharmacol.* 128: 169-181.

Crane, F.L. 1975. Oxidation of vanadium IV by cytochrome c oxidase: evidence for a terminal copper pathway. *Biochem. Biophys. Res. Comm.* 63: 355-361.

Crans, D.C., R.L. Bunch, and L.A. Theisen. 1989. Interaction of trace levels of vanadium(IV) and vanadium(V) in biological systems. *J. Am. Chem. Soc.* 111: 7597-7607..

Cutler, R.G. 1991. Antioxidants and aging. *Am. J. Clin. Nutr.* 53: 373 S-379 S.

Darr, D., and I. Fridovich. 1984. Vanadate and molybdate stimulate the oxidation of NADH by superoxide radical. *Arch. Biochem. Biophys.* 232: 562-565.

de Groot, H. and A. Littauer. 1989. Hypoxia, reactive oxygen, and cell injury. *Free Radical Biol. Med.* 6: 541-551.

Dix, T.A. and L.J. Marnett. 1983. Metabolism of polycyclic aromatic hydrocarbon derivatives to ultimate carcinogens during lipid peroxidation. *Science* 221: 77-79.

Eckardt, R.E. 1971. Petroleum fuel and airborne metals. *Arch. Environ. Health.* 23: 166-170.

Erdmann, E., W. Krawietz, G. Philipp, I. Hackbarth, W. Schmitz, and H. Scholz. 1979. Purified cardiac cell membranes with high $(\text{Na}^+ + \text{K}^+)$ ATPase activity contain significant NADH-vanadate reductase activity. *Nature (Lond.)* 282: 335-336.

Erdmann, E., W. Krawietz, F. Vyskocil, H. Dlouha, and J. Teisinger. 1981. Importance of cardiac cell membranes in vanadate-induced NADH oxidation. *Nature (Lond.)* 294: 288.

Erdmann, E., K. Werdan, W. Krawietz, W. Schmitz, and H. Scholz. 1984. Vanadate and its significance in biochemistry and pharmacology. *Biochem. Pharmacol.* 33: 945-950.

Finley, J.W. and M.S. Otterburn. 1993. The consequences of free radicals in foods. *Toxicol. Ind. Health.* 9: 77-91.

Fischer, E.H., H. Charbonneau, and N.K. Tonks. 1991. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* 253:401-406.

Fisher, G.L., K.L. McNeill, B.A. Prentice, and A.R. McFarland. 1983. Physical and biological studies

of coal and oil fly ash. *Environ. Health. Perspect.* 51: 181-186.

Flyger, H., F.P. Jensen, and K. Kemp. 1976. Air pollution in Copenhagen. Part I. Element analysis and size distribution of aerosols. *RISO-Rep.* (338): 1-70.

Goldsmith, A.H., K.W. Vorpahl, K.A. French, P.T. Jordan, and N.B. Jurinski. 1976. Health hazards from oil, soot and metals at a hot forging operation. *Am. Ind. Hyg. Assoc. J.* 37: 217-226.

Gower, J.D. 1988. A role for dietary lipids and antioxidants in the activation of carcinogens. *Free Radical Biol. Med.* 5: 95-111.

Haegele, A.D., S.P. Briggs, and H.J. Thompson. 1994. Antioxidant status and dietary lipid unsaturation modulate oxidative DNA damage. *Free Radical Biol. Med.* 16: 111-115.

Hajjar, J.J., S. Zakko, and T.K. Tomicic. 1986. Effect of vanadate on water and electrolyte transport in rat jejunum. *Biochim. Biophys. Acta* 863: 325-330.

Haque, S.J., V. Flati, A. Deb, B.R.G. Williams. 1995. Roles of protein-tyrosine phosphatases in stat1 α -mediated cell signaling. *J. Biol. Chem.* 270: 25709-25714.

Hauser, R., S. Elreedy, J.A. Hoppin, and D.C. Christiani. 1995. Upper airway response in workers exposed to fuel oil ash: nasal lavage analysis. *Occup. Environ. Med.* 52: 353-358.

Hecht, D., and Y. Zick. 1992. Selective inhibition of protein tyrosine phosphatase activities by H₂O₂ and vanadate in vitro. *Biochem. Biophys. Res. Comm.* 188: 773-779.

Heffetz, D., I. Bushkin, R. Dror, and Y. Zick. 1990. The insulinomimetic agents H₂O₂ and vanadate stimulate protein tyrosine phosphorylation in intact cells. *J. Biol. Chem.* 265: 2896-2902.

Hudson, T.G.F. 1964. Vanadium toxicology and biological significance. Elsevier Publ. Co., Amsterdam.

Hughes, M.F., W. Chamulitrat, R.P. Manson, and T.E. Eling. 1989. Epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene via a hydroperoxide-dependent mechanism catalyzed by lipoxygenases. *Carcinogenesis* 10: 2075-2080.

Ianzu, T., T. Taniguchi, S. Yanagi, and H. Yamamura. 1990. Protein-tyrosine phosphorylation and aggregation of intact human platelets by vanadate with H₂O₂. *Biochem. Biophys. Res. Comm.* 170: 259-263.

Jandhyala, B.S. and G.J. Hom. 1983. Physiological and pharmacological properties of vanadium. *Life Sci.* 33: 1325-1340.

Jaeschke, H. 1995. Mechanisms of oxidant stress-induced acute tissue injury. *Proc. Soc. Expl. Biol. Med.* 209: 104-111.

Kalogeropoulos, N., M. Scoullos, M. Vassilaki-Grimani, and A.P. Grimanis. 1989. Vanadium in particles and sediments of the northern Saronikos Gulf, Greece. *Sci. Total. Environ.* 79: 241-252.

Karimov, M.A., R.A. Doskeeva, E.K. Sarsebekov, K.D. Rakhimov, and A.A. Bel'khodzhaeva. 1988. Blastomogenic and nephrotoxic effects of a petroleum product obtained from high-vanadium oil at a temperature of 300-350 degrees C. [Rus.]. *Gig. Tr. Prof. Zabol.* (11): 53-55.

Karimov, M.A., R.A. Doskeeva, E.K. Sarsebekov, Z.D. Egorova, and G.V. Orlova. 1989. Vanadium level in the urine of workers on a shift-work schedule during drilling of oil wells for high-vanadium petroleum. [Rus.]. *Gig. Tr. Prof. Zabol.* (3): 9-12.

Karimov, M.A., R.A. Doskeeva, and E.K. Sarsebekov. 1991. Blastomogenic action of heavy vanadium-rich petroleum and its processing products. [Rus.] *Gig. Tr. Prof. Zabol.* (1): 18-20.

Kehrer, J.P. 1993. Free radicals as mediators of tissue injury and disease. *Crit. Rev. Toxicol.* 23: 21-48.

Klaassen, C.D. (ed). 1996. Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill, New York.

Kleinman, M.T., D.M. Bernstein, and T.J. Kneip. 1977. An apparent effect of the oil embargo on total suspended particulate matter and vanadium in New York City air. *J. Air. Pollut. Control Assoc.* 27: 65-67.

Kretzschmar, M. and H. Braunlich. 1990. Role of glutathione in vanadate reduction in young and mature rats: evidence for direct participation of glutathione in vanadate inactivation. *J. Appl. Toxicol.* 10: 295-300.

Kulkarni, A.P. and J.Z. Byczkowski. 1994a. Effects of transition metals on biological oxidations. In J.O. Nriagu and M.S. Simmons, Eds., *Environmental Oxidants*. John Wiley and Sons, Inc. New York, pp. 475-496.

Kulkarni, A.P. and J.Z. Byczkowski. 1994b. Hepatotoxicity. In E. Hodgson, P.E. Levi, (Eds.), *Introduction to Biochemical Toxicology*, Appleton & Lange, Norwalk, CT, pp. 459 - 490.

Lahav, M., H. Rennert, and D. Barzilai. 1986. Inhibition by vanadate of cyclic AMP production in rat corpora lutea incubated in vitro. *Life Sci.* 39: 2557-2560.

Lander, H.M., J.S. Ogiste, K.K. Teng, and A. Novogrodsky. 1995. p21^{ras} as a common signaling target of reactive free radicals and cellular redox stress. *J. Biol. Chem.* 270: 211295-21198.

Lees, R.E. 1980. Changes in lung function after exposure to vanadium compounds in fuel oil ash. *Br. J. Ind. Med.* 37: 253-256.

Lehninger, A.L. 1965. *The Mitochondrion: Molecular Basis of Structure and Function*. W.A. Benjamin, Inc., New York, pp. 109-114.

Levy, B.S., L. Hoffman, and S. Gottsegen. 1984. Boilermakers' bronchitis. Respiratory tract irritation associated with vanadium pentoxide exposure during oil-to-coal conversion of a power plant. *J. Occup. Med.* 26: 567-570.

Lin, Y-C. and F. Grinnell. 1995. Treatment of human fibroblasts with vanadate and platelet-derived growth factor in the presence of serum inhibits collagen matrix contraction. *Exp. Cell Res.* 221: 73-82.

Liochev, S. and I. Fridovich. 1988. Superoxide is responsible for the vanadate stimulation of NAD(P)H oxidation by biological membranes. *Arch. Biochem. Biophys.* 263: 299-304.

Liochev, S.I. and I. Fridovich. 1990. Vanadate-stimulated oxidation of NAD(P)H in the presence of biological membranes and other sources of O_2^- . *Arch Biochem. Biophys.* 279: 1-7.

Liochev, S.I. and I. Fridovich. 1996. Comments on vanadate chemistry. *Free Radical Biol. Med.* 20: 157.

Madany, I.M. and E. Raveendran. 1992. Polycyclic aromatic hydrocarbons, nickel and vanadium in air particulate matter in Bahrain during the burning of oil fields in Kuwait. *Sci. Total. Environ.* 116: 281-289.

Meyer, M., R. Schreck, and P.A. Baeuerle. 1993. H_2O_2 and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J.* 12: 2005-2015.

Moeller, R.B. Jr., V.F. Kalasinsky, M. Razzaque, J.A. Centeno, E.J. Dick, M. Abdal, I.I. Petrov, T.W. DeWitt, M. al Attar, J.M. Pletcher, and others. 1994. Assessment of the histopathological lesions and chemical analysis of feral cats to the smoke from the Kuwait oil fires. *J. Environ. Pathol. Toxicol. Oncol.* 13: 137-149.

Nechay, B.R. 1984. Mechanisms of action of vanadium. *Ann. Rev. Pharmacol. Toxicol.* 24: 501-524.

Nechay, B.R., L.B. Nanninga, and P.S.E. Nechay. 1986. Vanadyl (IV) and vanadate (V) binding to selected endogenous phosphate, carboxyl, and amino ligands; calculations of cellular vanadium species distribution. *Arch. Biochem. Biophys.* 251: 128-138.

North, P. and R.L. Post. (1984). Inhibition of (Na,K)-ATPase by tetravalent vanadium. *J. Biol. Chem.* 259: 4971-4978.

Nriagu, J.O. and J.M. Pacyna. 1988. Quantitative assessment of world-wide contamination of air, water and soil by trace metals. *Nature (Lond.)* 333: 134-139.

O'Flaherty, E.J. 1993. A pharmacokinetic model for chromium. *Toxicol. Lett.* 68: 145-158.

O'Flaherty, E.J. 1996. A physiologically based model of chromium kinetics in the rat. *Toxicol. Appl. Pharmacol.* 138: 54-64.

Papas, A.M. 1993. Oil-soluble antioxidants in foods. *Toxicol. Ind. Health.* 9: 123-149.

Parfett, C.L., and R. Pilon. 1995. Oxidative stress-regulated gene expression and promotion of morphological transformation induced in C3H/10T1/2 cells by ammonium metavanadate. *Food. Chem.*

Toxicol. 33: 301-308.

Paschoa, A.S., M.E. Warenn, N.P. Singh, E.W. Bruenger, S.C. Miller, M. Cholewa, and K.W. Jones. 1987. Localization of vanadium-containing particles in the lungs of uranium/vanadium miners. Biol. Trace. Element. Res. 13: 275-282.

Pistelli, R., N. Pupp, F. Forastiere, N. Agabiti, G.M. Corbo, F. Tidei, and C.A. Perucci. 1991. Increase of nonspecific bronchial reactivity after occupational exposure to vanadium. [Ital.] Med. Lav. 82: 270-275.

Pratt, D.E. 1993. Antioxidants indigenous to foods. Toxicol. Ind. Health. 9: 63-75.

Proffitt, R. and R.M. Case. 1984a. The effects of vanadate on ^{45}Ca exchange and enzyme secretion in the rat exocrine pancreas. Cell Calcium 5: 321-334.

Proffitt, R. and R.M. Case. 1984b. Vanadate stimulates rat pancreatic enzyme secretion through the release of calcium from an intracellular store. Cell Calcium 5: 335-340.

Pryor, W.A. 1993. Oxidative stress status measurement in humans and their use in clinical trials. In K. Yagi (Ed.), Active Oxygen, Lipid Peroxides, and Antioxidants, CRC Press, Boca Raton, FL, pp. 117-126.

Purucker, E. and H. Wernze. 1990. Hepatic efflux and renal extraction of plasma glutathione: marked differences between healthy subjects and the rat. Klin. Wochenschr. 68: 1008 - 1012.

Purushotham, K.R., P. Wang, and M.G. Humphreys-Beher. 1995. Effect of vanadate on amylase secretion and protein tyrosine phosphatase activity in the rat parotid gland. Mol. Cell. Biochem. 152: 87-94.

Ramanadham, S., C. Heyliger, M.J. Gresser, A.S. Tracey, and J.H. McNeil. 1991. The distribution and half-life for retention of vanadium in the organs of normal and diabetic rats orally fed vanadium(IV) and vanadium(V). Biol. Trace. Elem. Res. 30: 119-124.

Ramasarma, T. 1990. H_2O_2 has a role in cellular regulation. Indian J. Biochem. Biophys. 27: 269-274.

Rifkin, R.J. 1965. In vitro inhibition of $\text{Na}^+ - \text{K}^+$ and Mg^{2+} ATPases by mono, di, and trivalent cations. Proc. Soc. Exp. Biol. Med. 120: 802-803.

Ramasarma, T., W.C. MacKellar, and F.L. Crane. 1981. Vanadate-stimulated NADH oxidation in plasma membrane. Biochim. Biophys. Acta. 646: 88-98.

Roberfroid, M. and P.B. Calderon. 1994. Free Radicals and Oxidation Phenomena in Biological Systems. Marcel Dekker, Inc., New York, NY.

Rokhlin, O. and M.B. Cohen. 1995. Differential sensitivity of human prostatic cancer cell lines to the effects of protein kinase and phosphate inhibitors. Cancer Lett. 98: 103-110.

Rossi, B., G. Siciliano, C. Giraldi, C. Angelini, A. Marchetti, and P.L. Paggiaro. 1986. Toxic myopathy induced by industrial mineral oils: clinical and histopathological features. *Ital. J. Neurol. Sci.* 7: 599-604.

Russanov, E., H. Zaporowska, E. Ivancheva, M. Kirkova, and S. Konstantinova. 1994. Lipid peroxidation and antioxidant enzymes in vanadate-treated rats. *Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.* 107: 415-421.

Sadiq, M. and T.H. Zaidi. 1984. Vanadium and nickel content of Nowruz spill tar flakes on the Saudi Arabian coastline and their probable environmental impact. *Bull. Environ. Contam. Toxicol.* 32: 635-639.

Sarsebekov, E.K., B.U. Dzharbusynov, and R.A. Doskeeva. 1994. The nephrotoxic action of heavy crude with a high vanadium content and of its refinery products. [Rus.] *Urol. Nefrol. Mosk.* (3): 35-36.

Schiff, L.J. and J.A. Graham. 1984. Cytotoxic effect of vanadium and oil-fired fly ash on hamster tracheal epithelium. *Environ. Res.* 34: 390-402.

Shi, X., X. Sun, and N.S. Dalal. 1990. Reaction of vanadium(V) with thiols generates vanadium(IV) and thiyl radicals. *FEBS Letters* 271: 185-188.

Shimizu. T. 1995. Inhibitors of the dynein ATPase and ciliary or flagellar motility. *Methods Cell Biol.* 47: 497-501.

Sies, H. 1994. Oxidative stress: from basic research to clinical medicine. In A.E. Favier, J. Neve, P. Faure (Eds.), *Trace Elements and Free Radicals in Oxidative Diseases*. Aocs Press, Champaign, IL, pp. 1-7.

Simons, T.J.B. 1979. Vanadate - a new tool for biologists. *Nature (Lond.)* 281: 337-338.

Sohal, R.S. 1993. The free radical hypothesis of aging: an appraisal of the current status. *Aging Clin. Exp. Res.* 5: 3-17.

Sokolov, S.M. 1986. Methodological aspects of assessing atmospheric contamination with metal aerosols in the vicinity of thermal power complexes. *J. Hyg. Epidemiol. Microbiol. Immunol.* 30: 249-254.

Spatz, L. and A.D. Bloom. 1992. *Biological Consequences of Oxidative Stress: Implications for Cardiovascular Disease and Carcinogenesis*. Oxford Univ. Press., New York.

Srivastava, A.K., and J.-L. Chiasson. 1995. Vanadium Compounds: Biochemical and therapeutic applications. *Mol. Cel. Biochem.* 153: 1-240.

Staal, F.J.T., M.T. Anderson, G.E.J. Staal, L.A. Herzenberg, C. Gitler, and L.A. Herzenberg. 1994. Redox regulation of signal transduction: tyrosine phosphorylation and calcium influx. *Proc. Natl. Acad. Sci. USA.* 91: 3619-3622.

Stadtman, E.R., C.N. Oliver, P.E. Starke-Reed, and S.G. Rhee. 1993. Age-related oxidation reaction in proteins. *Toxicol. Ind. Health.* 9: 187-196.

Stankiewicz, P.J., A. Stern, and A.J. Davison. 1991. Oxidation of NADH by vanadium: kinetics, effects of ligands and role of H_2O_2 or O_2 . *Arch. Biochem. Biophys.* 287: 8-17.

Stohs, S.J. and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biol. Med.* 18: 321-336.

Stone, R.L. and J.E. Dixon. 1994. Protein-tyrosine phosphatases. *J. Biol. Chem.* 269: 31323-31326.

Svoboda, P., J. Teisinger, J. Pilar, and F. Vyskocil. 1984. Vanadyl (VO^{2+}) and vanadate (VO_3) ions inhibit the brain microsomal Na,K -ATPase with similar affinities, protection by transferrin and noradrenaline. *Biochem. Pharmacol.* 33: 2485-2497.

Tan, C.M., S. Xenoyannis, and R.D. Feldman. 1995. Oxidant stress enhances adenylyl cyclase activation. *Circ. Res.* 77: 710-717.

Tappel, A.L., A.A. Tappel, and C.G. Fraga. 1989. Application of simulation modeling to lipid peroxidation process. *Free Radical Biol. Med.* 7: 361-368.

Tipton, I.H. and J.J. Shaffer. 1964. Statistical analysis of lung trace element levels. *Arch. Environ. Health* 8: 56-67.

Todaro, A., R. Bronzato, M. Buratti, and A. Colombi. 1991. Acute exposure to vanadium-containing dusts: the health effects and biological monitoring in a group of workers employed in boiler maintenance. *[Ital.] Med. Lav.* 82: 142-147.

Troppens, H. 1969. Vanadium pentoxide poisoning. *[Ger.] Dtsch. Gesundheitsw.* 24: 1089-1092.

Vazquez, F., M. Sanchez, H. Alexander, and D. Delgado. 1991. Distribution of Ni, V, and petroleum hydrocarbons in recent sediments from the Veracruz coast, Mexico. *Bull. Environ. Contam. Toxicol.* 46: 774-781.

Videla, L.A., S.B.M. Barros, and V.B.C. Junquiera. 1990. Lindane-induced liver oxidative stress. *Free Radical Biol. Med.* 9: 169-179.

Vroegop, S.M., D.E. Decker, and S.E. Buxser. 1995. Localization of damage induced by reactive oxygen species in cultured cells. *Free Radical Biol. Med.* 18: 141-151.

Vyskocil, F., J. Teisinger, and H. Dlouha. 1980. A specific enzyme is not necessary for vanadate-induced oxidation of NADH. *Nature* 286: 516-517.

White, M.A., G.D. Reeves, S. Moore, H.A. Chandler, and H.J. Holden. 1987. Sensitive determination of urinary vanadium as a measure of occupational exposure during cleaning of oil fired boilers. *Ann. Occup. Hyg.* 31: 339-343.

Williams, G.M. 1993. Inhibition of chemical-induced experimental cancer by synthetic phenolic antioxidants. *Toxicol. Ind. Health.* 9: 303-308.

Yamaguchi, M., H. Oishi, S. Araki, S. Saeki, H. Yamane, N. Okamura, and S. Ishibashi. 1995. Respiratory burst and tyrosine phosphorylation by vanadate. *Arch. Biochem. Biophys.* 323: 382-386.

Yin, X., A.J. Davison, and S.S. Tsang. 1992. Vanadate-induced gene expression in mouse C127 cells: roles of oxygen derived active species. *Mol. Cell. Biochem.* 115: 85-96.

Younes, M., Kayser, E., and Strubelt, O. (1991). Effect of antioxidants on vanadate-induced toxicity towards isolated perfused rat livers. *Toxicology* 70: 141-149.

Younes, M., and O. Strubelt. 1991. Vanadate-induced toxicity towards isolated perfused rat livers: the role of lipid peroxidation. *Toxicology* 66: 63-74.

Ziegler, D.M. 1985. Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. *Ann. Rev. Biochem.* 54: 305-329.

Zychlinski, L. 1980. Toxicological appraisal of work-places exposed to the dust containing vanadium pentoxide. [Pol.]. *Bromat. Chem. Toksykol.* 8: 195-199.

Zychlinski, L., and J.Z. Byczkowski. 1978. Oxido-reduction properties of vanadium at rat liver mitochondria. 12th Fedn. Europ. Biochem. Soc. Meeting, Abstracts, 1720, Dresden, Germany.

Zychlinski, L., and J.Z. Byczkowski. 1990. Inhibitory effects of vanadium pentoxide on respiration of rat liver mitochondria. *Arch. Environ. Contam. Toxicol.* 19: 138-142.

Zychlinski, L., J.Z. Byczkowski, and A.P. Kulkarni. 1991. Toxic effects of long-term intratracheal administration of vanadium pentoxide in rats. *Arch. Environm Contam. Toxicol.* 20: 295-298.